

Serum Interleukin 6 Level and Nutrition Status as Potential Predictors of Clinical Leprosy Development Among Household Contacts in Endemic Areas

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Background. Leprosy is a chronic infectious disease that can lead to severe lifelong disabilities. Close contacts of patients with leprosy have a higher risk of acquiring the disease. Nevertheless, there is a lack of reliable markers to predict *Mycobacterium leprae* infection. We aimed to identify new potential markers for developing clinical leprosy among contacts.

Methods. Serum levels of interleukin (IL) 6, IL-8, IL-10, hemoglobin, ferritin, and transferrin saturation were measured in 67 patients with multibacillary leprosy (MB), 65 household contacts (HHCs) of MB patients, and 127 endemic controls (ECs). By means of multivariate logistic regression and receiver operating characteristic (ROC) analyses, we analyzed baseline variables and laboratory parameters that showed significant differences between MB in the HHC and EC groups and obtained the respective areas under the curve (AUC). Optimal cutoff values of the associated cytokines were also determined.

Results. Elevated IL-6 level was observed in MB patients compared to HHCs and ECs ($P = .022$ and $.0041$, respectively). Anemia and iron deficiency were also higher in the MB group compared to HHCs or ECs ($P < .001$). Likewise, we observed an increased risk of having MB leprosy in underweight HHCs (odds ratio [OR], 2.599 [95% confidence interval [CI], .991–6.820]) and underweight ECs (OR, 2.176 [95% CI, 1.010–4.692]). Further ROC analysis showed that high serum IL-6 level, underweight, anemia, and iron deficiency can discriminate leprosy from their HHCs (AUC, 0.843 [95% CI, .771–.914]; $P = .000$; optimal cutoff value of IL-6 = 9.14 pg/mL).

Conclusions. Our results suggest that serum IL-6 and nutrition status could serve as potential prognostic markers for the development of clinical leprosy in infected individuals.

Keywords. cytokines; disease development; household contact; leprosy; predictive marker.

Leprosy, caused by *Mycobacterium leprae*, is still a growing health threat with 202 185 new cases detected worldwide in 2019 [1]. Indonesia is still the third-highest contributor to leprosy per se with the largest proportion of multibacillary (MB) leprosy cases [1]. Household contacts (HHCs) of patients with leprosy, particularly the MB type, exhibit the highest risk of developing the disease [2–4]. Early case detection and contact management with prophylaxis are the current main strategies to control leprosy. However, the current laboratorial test used for selecting contacts who will receive prophylaxis still displays

low sensitivity (<40%) and therefore would miss more than half of future leprosy cases [5]. Identification and validation of sensitive markers for the progression of *M leprae* infection to clinical leprosy is imperative to break the chain of transmission and substantially reduce the new case detection rate toward leprosy elimination and possible eradication.

Leprosy manifests as a spectrum of clinical forms that greatly depends on the balance between inflammatory and anti-inflammatory immune responses against *M leprae*. This balance consists of a complex process involving innate immune cells (macrophages, dendritic cells, natural killer cells, keratinocytes), T-helper 1 (inflammatory) cytokines (interleukin [IL] 1, IL-6, IL-8, IL-12, interferon gamma [IFN- γ], tumor necrosis factor alpha [TNF- α]), and T-helper 2 (anti-inflammatory) cytokines (IL-4, IL-10). While the role of innate immune cells in the pathogenesis of leprosy cannot be neglected, current data suggest that T-cell responses through its cytokines determine the outcome of disease development. In the tuberculoid or paucibacillary (PB) form of leprosy, bacteria are rarely observed as the host has a strong cell-mediated immune response, whereas the humoral response dominates in the lepromatous or

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MB form with a high load of bacilli and therefore poses a higher risk of leprosy transmission [6]. Early detection of *M leprae* infection could therefore identify the main cytokines that distinguish individuals who are controlling bacterial replication from those who are developing the disease, particularly the MB type.

Several cytokines have been proposed as markers to identify individuals with leprosy. Nevertheless, there are very limited data regarding the markers discriminating between individuals with MB and those with PB leprosy. An in vitro study stimulating peripheral blood mononuclear cells (PBMCs) of patients with inactive lepromatous leprosy with sonicated *M leprae* extract and phorbol myristate acetate as a control demonstrated a significant increase of IL-6 and IL-8 levels in the *M leprae*-stimulated group [7]. Other studies [8, 9] also showed a significantly higher level of IL-6 in patients with lepromatous leprosy or MB leprosy compared to patients with tuberculoid leprosy and healthy controls, whereas IL-8 was suggested to play a pivotal role in cell recruitment in leprosy patients with disseminated mycobacterial infections (MB) in the absence of IFN- γ and TNF- α activation [10]. Furthermore, the AA genotype of IL-8 T-353A was observed as a risk factor for multibacillary leprosy (odds ratio [OR], 3.8 [95% confidence interval {CI}, 1.1–13.5]; $P = .023$), regardless of sex and age of disease onset [11]. Likewise, IL-10 polymorphism (819) TT and (-1082) GG genotypes were found to be significantly higher in patients with lepromatous leprosy compared to healthy controls [12]. A sustained IL-10 production can drive a permissive antimicrobial programming that leads to intracellular *M leprae* replication in patients with disseminated lepromatous or MB leprosy [13]. For the aforementioned reasons, it is noteworthy to investigate IL-6, IL-8, and IL-10 as potential predictors of MB leprosy disease development.

In addition to these cytokines, nutritional deficiencies have been suggested to impair host immune responses against *M leprae*. Several studies indicated nutritional deficiencies as a contributing factor to leprosy disease development [14–16]. Likewise, our previous study has shown that people who are anemic and underweight had a higher risk of contracting leprosy, and that low iron status was found more often in patients with leprosy compared to endemic controls (ECs) [17].

The aim of the present study was to investigate IL-6, IL-8, and IL-10 and nutritional status as potential markers for the development of clinical leprosy among contacts.

MATERIALS AND METHODS

Patient Consent Statement

This study was performed according to ethical standards in the Helsinki Declaration of 1964, as revised in 2008. Ethical approval for the study protocol was obtained from the institutional review board of the Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia (reference number 114 595/UN2.

F1/ETIK/2016). All of the participants comprising of patients with MB leprosy, their HHCs, and ECs were informed about the study objectives, the required amount and kind of samples, and their right to refuse to take part or withdraw from the study at any time without consequences for their treatment. A signed informed consent form was obtained from each participant prior to the start of the study.

Study Area and Population

This case-control study is part of a household-based MicroLep study that was conducted in rural areas of Bangkalan, a district of Madura, East Java, Indonesia, where leprosy is endemic [17]. During the study period, approximately 310 new cases were diagnosed in a total population of 1 million, yet no chemoprophylaxis therapy has been given to prevent leprosy in patient contacts.

Patients with MB leprosy between the ages of 18 and 65 were selected from the MicroLep database. HHCs who had been living with MB leprosy patients for at least a year and ECs who lived in the village or neighborhood with common characteristics as the cases were also selected. The following exclusion criteria were applied: paucibacillary leprosy, refusal to participate, limited understanding of information, pregnant or breastfeeding, and tested positive for helminth infections. Additionally, healthy controls who had any prior contact with individuals or household members with a history or newly diagnosed leprosy at the time of inclusion were also excluded.

Identification of Soil-Transmitted Helminth Infections

Diagnosis of helminth infection was made if a minimum of 1 ovum or larva was found in the fecal sample using the Harada-Mori paper strip culture method and formalin-ether sedimentation technique [18]. Based on the parasitological examination results, individuals who tested positive were treated with anthelmintic therapy and excluded from the study.

Blood Collection, Processing, and Storage

Peripheral blood samples were collected into ethylenediaminetetraacetic acid (EDTA) and serum separation tubes (SSTs) at inclusion. Samples in SSTs were allowed to clot for 30 minutes to 1 hour at ambient temperature (19°C–24°C) before spinning and separating. The serum samples of each participant were aliquoted into 1.5 mL Eppendorf tubes (Eppendorf, Milano, Italy); 1 aliquot per participant was kept at –80°C while the remaining serum and the whole blood with EDTA were used for measuring iron status and hemoglobin level.

Nutritional Status Assessment

Nutritional status was assessed based on body mass index (BMI) and the blood levels of hemoglobin, ferritin, iron, and total iron binding capacity (TIBC). Weight and height

were determined using a standardized portable scale (GEA Medical, Jakarta, Indonesia) and a standard measuring tape; the study participants were asked to remove their footwear and stand on a flat surface with their back against the wall. BMI was calculated by dividing weight (kilograms) with square of height (meters) and was defined underweight if $<18.5 \text{ kg/m}^2$ [19].

In regards to iron status, a diagnosis of iron deficiency was made when: (1) serum ferritin level $<30 \text{ } \mu\text{g/L}$ for HHCs and ECs [20]; (2) serum ferritin level $<100 \text{ } \mu\text{g/L}$ or transferrin saturation (TSAT) $<20\%$ for MB patients [21]. TSAT was calculated using the following formula: $\text{iron} / \text{TIBC} \times 100$. TSAT $<20\%$ is required to confirm iron deficiency if serum ferritin is $100\text{--}300 \text{ } \mu\text{g/L}$ [21].

Cytokine Measurement

We measured the levels of serum IL-6, IL-8, and IL-10 from all 3 subject groups using Luminex 200, a multiplex immunoassay kit with antibody-conjugated magnetic beads (R&D Systems). Serum samples were diluted and processed according to the manufacturer's instructions. Standard curves of known concentrations of recombinant human cytokines were used to convert fluorescence units into concentration units (picograms per milliliter). Values below the lower limit of quantification (LLOQ) were substituted with LLOQ/2 values. The samples were analyzed using the Varioskan LUX multimode microplate reader (Thermo Scientific), and the generated data were processed using the xPONENT software (Luminex).

Statistical Analysis

We performed statistical analysis using GraphPad Prism version 5.01 for Windows and IBM SPSS Statistics for Windows version 27.0. Qualitative variables were analyzed using 2×2 contingency tables and χ^2 test. Comparisons of the quantitative variables between the MB group with either the HHC or EC group were performed using t test or Mann-Whitney U test depending on the normality distribution of the data. To reduce the potential for known and unknown confounding variables, we also performed a multivariate logistic regression analysis on the baseline and laboratory data that showed significant differences in bivariate analysis between the leprosy and HHC groups. Receiver operating characteristic (ROC) analysis was performed and the areas under the curve (AUC) were obtained for significant markers between MB and ECs. The optimal cutoff value of the quantitative marker was obtained referring to a maximum Youden index and a modeling study by Blok et al [22] to determine the high- and low-risk groups. A P value of .05 was considered significant for all statistical analyses.

RESULTS

Participant Characteristics

At baseline, there were 401 participants recruited in the MicroLep study, consisting of 100 patients with leprosy (11 PB and 89 MB), 101 HHCs, and 200 ECs. After exclusion of 142 participants (110 helminth-positives, 11 PB patients, 11 HHCs of PB patients, and 10 with insufficient serum and/or fecal samples), a total of 259 participants consisting of 67 MB patients, 65 HHCs, and 127 ECs were included in the study. General characteristics, hemoglobin, iron profile, and cytokine

Table 1. General Characteristics and Laboratory Parameters of the Study Population

Variable	Total Participants (n = 259)	Leprosy Patients (n = 67)	Household Contacts (n = 65)	Endemic Controls (n = 127)
Mean age, y	39.3	40.6	37.3	39.6
Sex, No. (%)				
Male	127 (49.0)	34 (50.7)	23 (35.4)	70 (55.1)
Female	132 (51.0)	33 (49.3)	42 (64.6)	57 (44.9)
Body mass index				
Mean \pm SEM, kg/m ²	22.6 \pm 0.3	21.2 \pm 0.5	23.1 \pm 0.6	23.2 \pm 0.4
Underweight, No. (%)	39 (15.1)	16 (23.9)	7 (10.8)	16 (12.6)
Normal, No. (%)	118 (45.6)	32 (47.8)	31 (47.7)	55 (43.3)
Overweight/obese, No. (%)	102 (39.4)	19 (28.3)	27 (41.5)	56 (44.1)
Laboratory value				
Hemoglobin, mean \pm SEM	13.3 \pm 0.1	11.8 \pm 0.3	13.4 \pm 0.2	14.0 \pm 0.1
Serum iron, mean \pm SEM	85.1 \pm 2.0	71.9 \pm 4.1	84.6 \pm 3.8	92.3 \pm 2.7
TIBC, mean \pm SEM	284.8 \pm 2.9	270.8 \pm 6.1	293.9 \pm 6.2	287.6 \pm 3.6
Ferritin, median (IQR)	103.2 (60.3–161.4)	134.1 (68.7–258.3)	88.5 (45.2–149.4)	100.7 (73.0–151.7)
TSAT, mean \pm SEM	30.3 \pm 0.7	26.6 \pm 1.3	29.6 \pm 1.5	32.7 \pm 1.0
IL-6, median (IQR)	5.7 (3.7–10.0)	9.2 (4.5–19.9)	5.6 (3.4–10.3)	5.6 (3.8–8.0)
IL-8, median (IQR)	55.4 (17.0–120.1)	62.3 (31.9–127.9)	57.5 (16.5–120.1)	45.6 (12.7–109.1)
IL-10, median (IQR)	13.3 (13.3–24.4)	14.0 (13.3–30.0)	13.3 (13.3–24.4)	13.3 (9.8–19.3)

Abbreviations: IL, interleukin; IQR, interquartile range; SEM, standard error of the mean; TIBC, total iron binding capacity; TSAT, transferrin saturation.

levels of the participants are shown in [Table 1](#). The majority of all participants were normoweight (118/259 [45.6%]), with a mean age of 39.3 years and no observed difference between sex.

Although the HHC group had a relatively higher proportion of female participants and younger mean age compared to the MB and EC groups, there were no statistically significant differences with respect to age and sex.

Nutritional Status

BMI distribution was significantly different across the groups (mean difference, -1.875 [95% CI, -3.371 to $-.381$; $P < .05$] for MB vs HHCs and -1.948 [95% CI, -3.309 to $-.588$; $P < .05$] for MB vs ECs). The risk of having MB leprosy was also increased in underweight participants (OR, 2.599 [95% CI, .991–6.820; $P < .05$] in HHCs and 2.176 [95% CI, 1.010–4.692; $P < .05$] in ECs).

In addition to low BMI, participants in the MB group had a significantly lower hemoglobin, serum iron, and transferrin levels compared with HHCs or ECs ([Table 1](#)). Likewise, the risk of contracting leprosy is higher in those with anemia (OR, 7.083 [95% CI, 2.930–17.126; $P < .001$] in HHCs and OR, 10.771 [95% CI, 4.926–23.552; $P < .001$] in ECs) and iron deficiency regardless of the presence of anemia (OR, 11.42 [95% CI, 4.340–30.047; $P < .001$] in HHCs and OR, 17.274 [95% CI, 7.295–40.904; $P < .001$] in ECs).

Serum Cytokine Levels

To evaluate the potential markers in the development of leprosy, we compared the expression of cytokines between MB with either HHCs or ECs. The median values of IL-6 level were significantly higher in the MB group (8.70 pg/mL [interquartile range {IQR}, 4.34–19.43]) compared to HHCs (5.57 pg/mL [IQR, 3.16–10.18]) or ECs (5.62 pg/mL [IQR, 3.70–8.20]) ($P = .022$ and $.0041$, respectively). Meanwhile, IL-8 and IL-10 levels were significantly elevated in the MB group (60.41 pg/mL [IQR, 31.44–120.30] and 13.67 pg/mL [IQR, 13.26–29.18]) compared to ECs (41.17 pg/mL [IQR, 11.29–102.60] and 13.26 pg/mL [IQR, 9.64–19.34]) ($P = .0394$ and $.0233$, respectively), yet did not show any significant difference when compared to HHCs (56.12 pg/mL [IQR, 16.30–108.30] and 13.26 pg/mL [IQR, 13.26–21.25]) ([Figure 1](#)).

Associations of IL-6 and Markers of Nutritional Status

Among the 3 investigated cytokines, IL-6 is the only cytokine that significantly different between MB group with either HHCs or ECs. Subsequently, we performed association analysis on this cytokine with the laboratory markers of nutritional status measured in this study. We observed that people with higher IL-6 levels have an increased risk of having anemia (OR, 3.715 [95% CI, 1.975–6.989]; $P < .001$) and iron deficiency regardless of the presence of anemia (OR, 2.782 [95% CI, 1.464–5.284]; $P = .001$).

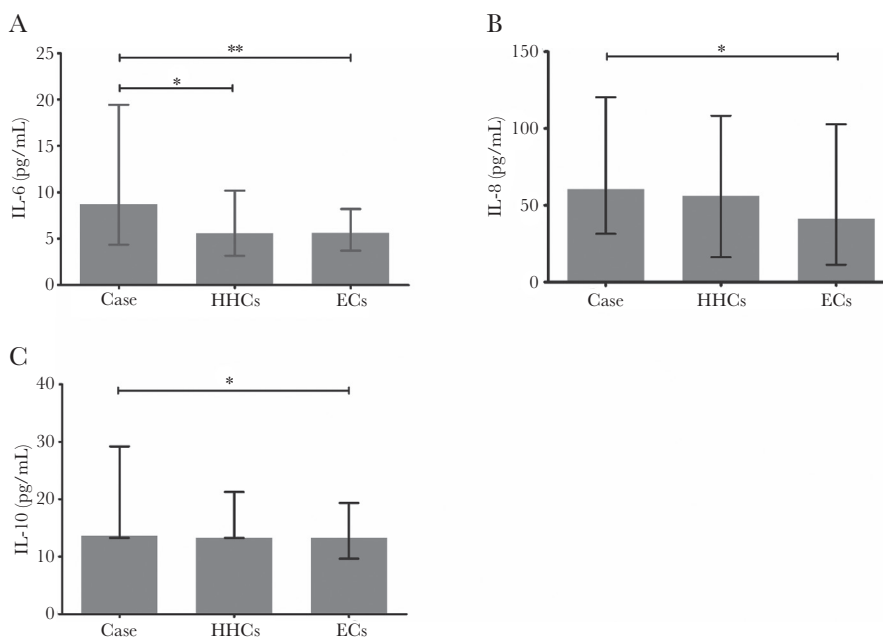


Figure 1. Interleukin (IL) 6, IL-8, and IL-10 levels among patients with multibacillary leprosy (Case), household contacts (HHCs), and endemic controls (ECs). * $P < .05$; ** $P < .01$.

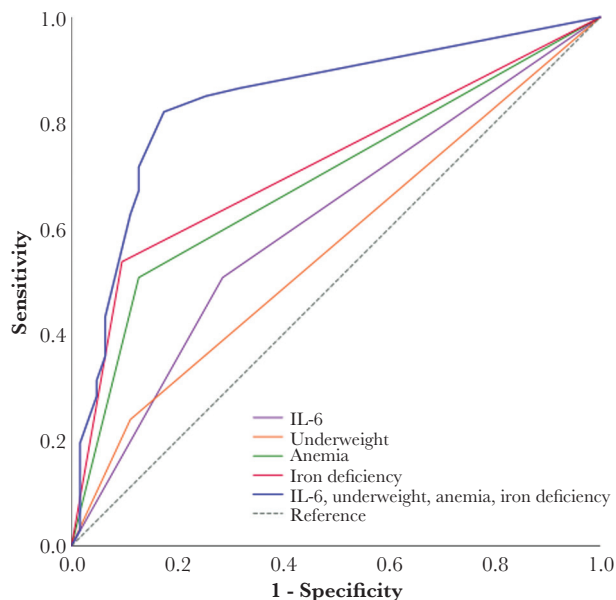


Figure 2. Receiver operating characteristic curves for interleukin 6 (IL-6), underweight, anemia, and iron deficiency between the multibacillary leprosy and household contact groups.

Multivariate Analysis

To reduce the potential for known and unknown confounding variables, we performed a multivariate logistic regression analysis on IL-6 between MB and HHCs with BMI as a covariate. In the BMI adjusted model, the difference of IL-6 levels between the groups remains significant with a P value of .028. ROC analysis was performed, showing AUC of 0.617 (95% CI, .520–.714); $P = .022$). This result indicates that IL-6 could provide discrimination between MB and HHCs (Figure 2). Furthermore, the cutoff value corresponding to a maximum Youden index was derived to determine the high- and low-risk groups with reference to IL-6. The cutoff obtained for IL-6 was 13.42 pg/mL corresponding to a maximum index of 1.26 (sensitivity, 36.36%; specificity, 90.63%). Based on the given cutoff of 13.42 pg/mL, the probability that a person who has serum IL-6 level above the cutoff having a disease is 0.80. In addition to Youden index, a modeling study by Blok et al [22] demonstrated that a test for subclinical leprosy with a sensitivity of at least 50% could substantially reduce *M leprae* transmission and effectively reduce the new case detection rate in short run. Accordingly, the cutoff for this hypothesis would be 9.14 pg/mL (sensitivity, 50%; specificity, 73.44%).

In the bivariate analysis, the MB group was also shown to have a significantly higher number of participants with underweight, anemia, and iron deficiency. Hence, we also evaluated the HHC group for risk of disease based on the presence of these variables along with IL-6 values, referring to the respective cutoff that was previously determined. We obtained an AUC of 0.843 (95% CI, .771–.914) and $P = .000$ (Figure 2).

DISCUSSION

In the present study, we evaluated serum IL-6, IL-8, and IL-10 levels and nutrition status as risk markers of clinical leprosy development. Our results suggest that serum IL-6, underweight, anemia, and iron deficiency are potential markers for the development of leprosy among individuals who have close contact with index cases or live in leprosy-endemic areas.

IL-6 is a soluble mediator with a pleiotropic effect on inflammation, immune response, and hematopoiesis [23, 24]. After IL-6 is produced in a local lesion in the initial stage of inflammation, it circulates and induces synthesis of acute phase proteins such as C-reactive protein, serum amyloid A protein, fibrinogen, and hepcidin, and reduces the production of fibronectin, albumin, and transferrin [25–27]. More data in recent years also indicate regulation of T-cell differentiation and activation as well as promotion of antibody production by B cells as another key fields of action of IL-6 [25, 26]. Up to now, serum IL-6 has not been specifically addressed as the responsible cytokine for the disease development of leprosy. Nevertheless, the associations of the aforementioned IL-6 actions with leprosy have been demonstrated in previous studies [28–30]. As the main regulator of hepcidin, IL-6 modulates cellular iron export through ferroportin to plasma and extracellular fluid. Ferroportin is expressed on duodenal enterocytes (for dietary iron absorption), macrophages in liver and spleen (for recycling of old erythrocytes), hepatocytes (for iron storage), and placental trophoblasts (for iron transfer to the fetus). High hepcidin level blocks ferroportin-mediated iron export from the gut and macrophage that leads to iron restriction erythropoiesis and anemia with chronic inflammation [31–33]. Abundant iron deposit in macrophages is fully available for *M leprae* intracellular growth [34, 35], and may contribute to defective capacity of *M leprae*-infected macrophages to respond to activating signals as demonstrated in previous studies [36–38]. This is also supported by the higher expression of hepcidin level [39] and lower level of ferroportin [34] found in patients with lepromatous leprosy [27]. A previous study by our group [17] observed decreased serum iron levels in patients with leprosy compared to ECs and observed that people with anemia have an increased risk of contracting leprosy per se (OR, 4.01 [95% CI, 2.10–7.64]; $P = .000$). The present study with MB leprosy also reported similar findings, with ORs of 7.083 in HHCs and 10.771 in ECs for anemia, and ORs 11.42 in HHCs and 17.274 in ECs for iron deficiency regardless of the presence of anemia. Further association analyses showed that people with higher IL-6 levels have an increased risk of having anemia (OR, 3.715 [95% CI, 1.975–6.989]; $P < .001$) and iron deficiency regardless of the presence of anemia (OR, 2.782 [95% CI, 1.464–5.284]; $P = .001$), suggesting the interplay between IL-6 with anemia and iron status. Additionally, other IL-6 actions that have been demonstrated in leprosy were AA amyloidosis [28, 29] and procoagulant status

due to high levels of plasmatic fibrinogen, anticardiolipin antibodies, von Willebrand factor, and soluble tissue factor [30]. The high level of fibrinogen may also promote the development of fibrosis in several organs, including skin and nerve, that tips the balance between healthy wound healing and irreversible fibrotic scarring in the form of chronic ulcers and deformities [40]. IL-6 can be a double-edged sword for the host; while an immediate and transient expression of IL-6 activates host defense mechanisms to remove the source of stress, uncontrolled and persistent IL-6 production may contribute to chronic inflammation and iron deficiency that lead to the development of various diseases, including leprosy. Collectively, our data, together with the aforementioned studies, support the hypothesis of IL-6 involvement in the pathogenesis of leprosy and indicate that a deficiency in essential nutrients that are needed to support an adequate immune response against infectious agents could increase the risk of contracting clinical leprosy.

The present study showed that groups with IL-6 level above the cutoff values have higher probability to develop clinical leprosy, which increase more if they also have low BMI, anemia, and iron deficiency. Two cutoff values were obtained according to the maximum Youden index and a modeling study by Blok et al [22]. The former has high specificity, yet very low sensitivity (<50%). A high-specificity test is useful for ruling in people who actually have the disease and will not generate many false-positive results in healthy individuals. Nevertheless, sensitivity is essential to identify infected individuals who will likely progress to disease and therefore enable the possibility for prevention. A minimum sensitivity of 50% is substantial to effectively reduce the new case detection rate of leprosy, and a 3-year follow-up for individuals testing negative could reach a similar impact as a test with a sensitivity of 100% [22]. According to this model, we obtained an optimal serum IL-6 cutoff value of 9.14 pg/mL with sensitivity of 50% and specificity of 73.44%.

In addition to IL-6, we also observed a significant association of IL-8 and IL-10 with multibacillary leprosy. These results are consistent with previous studies that demonstrated the influence of IL-8 and IL-10 responses in the disease progression to MB leprosy [10, 11]. However, we did not perform the ROC analysis on IL-8 and IL-10 as we did not observe significant differences of their serum levels across the groups of patients with MB leprosy and their HHCs.

This study has several limitations. The first limitation is that we did not include patients who had PB leprosy due to the lower risk of transmission to the community and the small PB/MB ratio in Indonesia (15/85). The impacts of this test in areas with different PB/MB ratios might be different and therefore need further validation. Second, we only included limited nutritional markers in this study. While the role of other biomarkers for other nutrients should also be analyzed, we only focused on anemia and iron profiles that have been proven to correlate with dietary intake and are more affordable to most patients with leprosy.

CONCLUSIONS

The results presented in this study suggest the association of IL-6, IL-8, and IL-10 with leprosy. Furthermore, our results suggest that serum IL-6 could serve as a potential marker to identify HHCs who are at an increased risk of developing clinical leprosy. This risk is even higher in the presence of low nutrition status (underweight, anemia, and iron deficiency), indicating the importance of considering the individual's clinical characteristics along with IL-6 level to determine the prognosis of *M leprae* infection among close contacts of patients with leprosy. Further study evaluating the in vitro production of IL-6 by PBMCs of patients with leprosy (PB and MB), their HHCs, and healthy controls from both endemic and nonendemic areas following the stimulation with *M leprae*-specific epitopes [41] could aid in confirming our findings.

Notes

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All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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